

Prevalence of mould in chicken meat-cuts, giblets and products with immuno-affinity detection of aflatoxin residues

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Abstract

Mould contamination and aflatoxin residues are considered big problems in the food chain, therefore 140 random samples of frozen chicken breast, thigh, gizzard, heart, liver, chicken burger, and chicken luncheon (twenty of each) were examined for detection of mould contamination level there proteolytic and lipolytic activity and aflatoxin residues in examined products. Mould colonies were detected in 100% and 30% of samples with mean values of 3.42 ± 0.71 and $1.29 \pm 0.30 \log_{10} \text{CFU/g}$ in the chicken burger and chicken luncheon, respectively. The identified species were *Aspergillus* 76/140 (54.3%), *Penicillium* 39/140 (27.9%), *Alternaria* 35/140 (25%), *Cladosporium* 30/140 (21.4%), *Rhizopus* 13/140 (9.3%), *Acremonium* 10/140 (7.1%), *Mucor* 9/140 (6.4%), *Fusarium* 7/140 (5%) and *Sporotrichum* 7/140 (5%). *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, *Aspergillus ochraceus* and *Aspergillus terreus* were identified with total percentages of 53(37.9%), 25(17.9%), 21(15%), 5(3.5%), 5(3.5%) and 8(5.7%), respectively. The proteolytic and lipolytic activity detected in all species ranged between high, moderate and weak degrees except *A. parasiticus* shows only lipolytic activity. The examined samples had variable incidence and concentrations of aflatoxin residues as (20%) 1.49 ± 0.53 , (15%) 1.89 ± 0.89 , (55%) 8.79 ± 14 , (25%) 1.64 ± 0.38 , (20%) 2.3 ± 0.72 , (65%) 9.21 ± 1.12 and (35%) $4.12 \pm 0.68 \mu\text{g/kg}$ in frozen chicken breast, thigh, gizzard, heart, liver, chicken burger and chicken luncheon, respectively. Thus, strict hygienic measures during the production of chicken products must be adopted to minimize the deviation in their nutritional quality due to the growth of proteolytic and lipolytic mould, in addition, to protecting human health from the hazards of aflatoxin residues.

1. Introduction

The consumption of chicken meat products increased worldwide due to the high content of essential amino acids, and their competitive price. Chicken meat is considered a significant source of protein in Egypt due to the lack of red meat production (Hussein *et al.*, 2018). Mould contamination of chicken meat products indicated bad sanitary and hygienic conditions adopted during the production cycle. Moreover, the growth of mould on the chicken product surfaces results in high economic losses and characterizes a hazard to human health due to the opportunity of aflatoxin production. Meat additives, such as spices, added during the formulation of chicken products can significantly increase the mould contamination of the chicken products (Gourama and Bullerman, 1995). The occurrence of aflatoxin in meat

additive collected from Egypt at concentrations 2.01 ± 0.45 , 4.14 ± 1.17 , 0.64 ± 0.24 and 0.47 ± 0.22 ppb for red pepper, nutmeg, cumin and black pepper, respectively (El-Ghreeb *et al.*, 2013). Furthermore, detected in luncheon, hot dog and corned beef with mean values of 10.14 ± 0.39 , 11.5 ± 0.63 and 10.16 ± 0.43 ppb, respectively (Algahtani *et al.*, 2020). Enzymatic degradation is a natural process that occurs in meat after ageing and is enhanced due to temperature abuse (Tauro *et al.*, 1986). Mould commonly produces extracellular proteases and lipases. Proteases (peptidases or proteolytic enzymes) can initiate and catalyse the hydrolysis of peptide bonds in protein. The cleavages of peptide bonds result in the degradation of protein into their original amino acids (Sabotič and Kos, 2012). Lipases have been studied for a number of moulds, primarily hyphomycetes, Zygomycetes, and yeasts. In

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meat, fat hydrolysis can occur enzymatically or non-enzymatically. The enzymatic hydrolysis of fats is termed fat deterioration (lipolysis) and is governed by lipases, esterases and phospholipase (Ghaly *et al.*, 2010). Mycotoxicosis, which occurs in food as a result of the presence of *A. flavus*, is an example of "poisoning by natural means," and is therefore analogous to pathologies caused by heavy metals or pesticide residues. The clinical picture of mycotoxicosis mainly depends on the amount and duration of the exposure, type of mycotoxin, the age and health condition of the exposed person, genetics factors, dietary status, and interface with other toxic substances (Bennett and Klich, 2003). *Aspergillus flavus* and *A. parasiticus* fungi are mainly incriminated in Aflatoxins (AFs) production which defined as toxic metabolites, naturally occurring in food. AFs have been identified since the 1960s as significant contaminants by the agricultural production community and control strategies (Guo *et al.*, 2000). *Aspergillus flavus* is responsible for the production of aflatoxin B1 and B2 (Wilson *et al.*, 2002). Aflatoxin B1 has been classified as a "group 1 carcinogen", affecting the liver by International Agency for Research on Cancer (IARC, 1993). Once aflatoxin contaminates food cooking methods like Steaming as well as boiling caused a none significant reduction of aflatoxin level (Diedhiou *et al.*, 2012). Thus, this study was conducted to estimate the prevalence of mould contamination in chicken meat cuts, giblets and products. Furthermore, identification of prevalent mould genera and their proteolytic and lipolytic activity was carried out. In addition, to the detection of aflatoxin residues in examined samples.

2. Materials and methods

2.1 Samples collection and preparation

A total of 140 random samples in frozen state -18°C represented by chicken breast, thigh, gizzard, heart, liver, frozen chicken burger and cooked chicken luncheon stored at 4°C (twenty of each) were collected from different localities in Zagazig city, Egypt. Samples were identified, packed then transferred refrigerated immediately to the Food Control Department Faculty of Veterinary Medicine Zagazig University, Egypt where mycological examinations were carried out. From each sample, 25 g were homogenized in 225 mL of sterile buffered peptone water 0.1% (APHA, 2001).

2.2 Determination of the total mould count

Total mould count (TMC) was estimated by culturing on duplicate plates of malt extract agar (Oxoid, MED 030) and Czapeck-Dox agar (HiMedia, M075). The plates were incubated at 25°C for 5-7 days in the dark. The incubated plates were examined daily for the

star shape mould growth, which is picked up under complete aseptic conditions with its surrounding medium and transferred into malt extract agar slope for further examination. TMC Estimated by counting both mould colonies on acidified malt extract agar and osmophilic moulds on Czapeck-Dox agar.

2.3 Identification of isolated mould

Mould is identified into genera depending on their micromorphological properties. In brief, the preserved isolates were subcultured on Czapeck- Dox agar and malt extract agar then, incubated at 25°C for 5-7 days. The identification of obtained colonies was carried out by observation and measurements of the macroscopical and microscopical characteristics carefully for identification according to Pitt and Hocking (2009).

2.4 Evaluation of proteolytic and lipolytic activity of isolated mould

Mould colonies cultivated on casein hydrolysis medium at 30°C for 7 days for estimation of proteolytic activity. Briefly, casein hydrolysis medium supplemented with skim milk (HIMEDIA, M763), which gives an opaque medium due to acid production, indicated by a clear zone surrounding the mould colony according to Paterson and Bridge, (1994). The lipolytic activity of mould was carried out on Tween 80 for 7 days at 30°C according to Ullman and Blasins, (1974). Positive lipolytic activity was noticed as opaque zone adjoining mould colony due to liberation of oleic acid by mould enzyme that, interact with calcium salt crystals.

2.5 Quantitative detection of total aflatoxins

Total aflatoxins (B1+B2+G1+G2) estimated by fluorometer (VICAM. Series 4) twenty-five grams of ground samples with five grams of NaCl were extracted in hundred mL methanol: water (80:20) three times. The extracts were diluted four times with bi-distilled water and filtrated using glass microfibre filter, only four mL of filtrated extract at a rate of 1-2 drops/second passed through (AflaTest®-P affinity column). One mL of HPLC grade methanol was used for elution of affinity column at a rate of one to two drops/second and finally collected in a glass cuvette. The AflaTest developer added 0.1 mL and mixed it well with the content of the cuvette then put the mixture in calibrated fluorometer followed by a reading of aflatoxin level after 1 min. The limit of detection ranged from 0.1 ppb to 300 ppb.

2.6 Statistical analysis

Mould counts were translated to CFU/g using base-10 logarithms. After confirming normality with Shapiro-test, Wilk's data was analysed using the one-way

ANOVA method of SPSS v.23 (SPSS Inc., Chicago, Illinois, USA). To see if there were any major variations between mean values, Tukey's multiple comparison tests were used. The variance in the data was expressed as means s.e.m., and the alpha level for evaluating significance was set at 0.05.

3. Result and discussion

3.1 Mould count in examined samples

Chicken meat may be contaminated with mould during slaughtering of the birds, transportation, or processing by the use of contaminated utensils or contaminated additives and spices, which are considered the most important source of mould contamination in chicken products (Jay *et al.*, 2005). Moreover, inadequate cold preservation of chicken meat, giblets, and products may result in mould growth, especially, if the initial microbial load is high (Morshedy and Sallam, 2009). The mould colonies detected in all examined samples the highest percentage belongs to the frozen burger (100%) and the lowest percentage was the share of luncheon (30%) (Table 1). Mould previously detected within different percentages (41%) of sausage samples collected from Italy (Iacumin *et al.*, 2009); (65.7%) and (34.2%) for fresh and frozen chicken cuts collected from Nigeria (Ogu *et al.*, 2017); (45%) meat, (50%) minced meat, (62.5%) luncheon, (80%) burger and (82.5%) sausage samples collected from Egypt (Habashy *et al.*, 2019). The existence of mould in most samples possibly suggests contamination of the environment where moulds are ubiquitous in air, soil, water, feeds and processing materials (Greco *et al.*, 2014). Regarding total mould count in descending manner burger, gizzard, liver, heart, breast, thigh and luncheon contained 3.42 ± 0.71 ,

2.89 ± 0.68 , 2.85 ± 0.62 , 2.23 ± 0.46 , 1.92 ± 0.42 , 1.84 ± 0.39 and 1.29 ± 0.30 \log_{10} CFU/g, respectively (Table 1). Burger samples were significantly higher contaminated than other samples ($p < 0.05$) which attributed to the mixing of mould contaminated raw materials (chicken cuts, breading flours, starch and spices) that survive through processing steps. The obtained counts are nearly similar to Vural *et al.* (2013) (1- 4.3 \log_{10} CFU/g) in frozen turkey collected from Turkey; Ogu *et al.* (2017) (2.11 – 4.34 \log_{10} CFU/g) in fresh and frozen chicken collected from Nigeria.

3.2 Mould identification

Nine mould genera were isolated from examined samples in this study namely Aspergillus, Penicillium, Alternaria, Cladosporium, Rhizopus, Acremonium, Mucor, Fusarium, Sporotricum (Table 2). Aspergillus and Penicillium species were the commonly identified 18 (90%) and 9 (45%), 15 (75%) and 8 (40%), 12 (60%) and 6 (30%), 11 (55%) and 7 (35%), 9 (45%) and 5 (25%), 8 (40%) and 3 (15%), 3 (15%) and 1 (5%) in burger, liver, thigh, gizzard, breast, heart, luncheon, respectively (Table 2). Our finding substantiates with Darwish *et al.* (2016), Ogu *et al.* (2017) and Habashy *et al.* (2019) found that Aspergillus and Penicillium were the common mould genera isolated from chicken and meat samples. The frequent isolation of these two moulds attributed to the growth ability over pH ranged from 2 to 11, the temperature ranged from -10 to 60°C, water activity ranged from 0.620 to 0.995 and wide range of nutrient availability (Pitt and Hocking, 2009). The other identified species were Alternaria 25%, Cladosporium 21.4%, Rhizopus 9.3%, Sporotricum 7.1%, Mucor 6.4%, Acremonium 5% and Fusarium 5% (Table 2). The isolated moulds in this study are usually

Table 1. Prevalence and count of mould (\log_{10} CFU/g) of examined chicken meat, giblets and products (n = 20).

	Breast	Thigh	Liver	Heart	Gizzard	Burger	Luncheon
Prevalence	12 (60%)	14 (70%)	17 (85%)	13 (65%)	12 (60%)	20 (100%)	6 (30%)
Minimum	1.2	1	1.3	1	1.4	2.3	1
Maximum	2.8	2.9	3.4	3.2	3.8	4.3	1.8
Mean±SD	1.92 ± 0.42^{bc}	1.84 ± 0.39^{bc}	2.85 ± 0.62^b	2.23 ± 0.46^{bc}	2.89 ± 0.68^b	3.42 ± 0.71^a	1.29 ± 0.30^c

Values are presented as mean±SD. Values with different superscripts within each row are significantly different ($P < 0.05$).

Table 2. Prevalence of mould genera in examined chicken meat, giblets and products.

	Breast	Thigh	Liver	Heart	Gizzard	Burger	Luncheon	Total
Aspergillus	9 (45%)	12 (60%)	15 (75%)	8 (40%)	11 (55%)	18 (90%)	3 (15%)	76 (54.3%)
Penicillium	5 (25%)	6 (30%)	8 (40%)	3 (15%)	7 (35%)	9 (45%)	1 (5%)	39 (27.9%)
Alternaria	4 (20%)	8 (40%)	6 (30%)	2 (10%)	5 (25%)	8 (40%)	2 (10%)	35 (25%)
Cladosporium	5 (25%)	3 (15%)	7 (35%)	4 (20%)	3 (15%)	7 (35%)	1 (5%)	30 (21.4%)
Rhizopus	2 (10%)	-	3 (15%)	1 (5%)	4 (20%)	3 (15%)	-	13 (9.3%)
Sporotricum	2 (10%)	-	3 (15%)	1 (5%)	-	4 (20%)	-	10 (7.1%)
Mucor	-	1 (5%)	2 (10%)	1 (5%)	2 (10%)	3 (15%)	-	9 (6.4%)
Acremonium	-	2 (10%)	3 (15%)	-	-	2 (10%)	-	7 (5%)
Fusarium	-	2 (10%)	1 (5%)	-	3 (15%)	1 (5%)	-	7 (5%)

Table 3. Prevalence of *Aspergillus* species in examined chicken meat, giblets and products.

	Breast	Thigh	Liver	Heart	Gizzard	Burger	Luncheon	Total
<i>A. niger</i>	6 (30%)	7 (35%)	11 (55%)	5 (25%)	9 (45%)	13 (65%)	2 (10%)	53(37.9%)
<i>A. flavus</i>	3 (15%)	2 (10%)	6 (30%)	2 (10%)	4 (20%)	6 (35%)	2 (10%)	25(17.9%)
<i>A. fumigatus</i>	1 (5%)	3 (15%)	4 (20%)	3 (15%)	4 (20%)	5 (25%)	1 (5%)	21(15%)
<i>A. parasiticus</i>	-	-	-	-	2 (10%)	3 (15%)	-	5(3.5%)
<i>A. ochraceus</i>	-	-	2 (10%)	-	1 (5%)	2 (10%)	-	5(3.5%)
<i>A. terrus</i>	-	1 (5%)	2 (10%)	-	1(5%)	4(20%)	-	8(5.7%)

Table 4. Lipolytic and proteolytic activities of the isolated mould.

	No. of tested	proteolytic				Lipolytic			
		Positive	High	Moderate	weak	Positive	High	Medium	Weak
<i>A. niger</i>	53	42	11	29	2	46	44	2	0
<i>A. flavus</i>	25	21	21	0	0	25	25	0	0
<i>A. fumigatus</i>	21	13	11	2	0	16	1	5	10
<i>A. parasiticus</i>	5	0	0	0	0	0	0	0	0
<i>A. ochraceus</i>	5	4	0	1	3	4	0	2	2
<i>A. terrus</i>	8	6	0	1	5	5	0	1	4
<i>Penicillium</i> sp.	39	39	21	2	16	39	6	31	2
<i>Alternaria</i> sp.	35	35	0	0	35	35	2	4	29
<i>Cladosporium</i> sp.	30	22	0	18	4	30	0	2	28
<i>Rhizopus</i> sp.	13	13	0	0	13	13	1	2	10
<i>Sporotricum</i> sp.	10	4	0	1	3	7	0	3	4
<i>Mucor</i> sp.	9	9	0	0	9	9	0	2	7
<i>Acremonium</i> sp.	7	5	0	2	3	7	2	3	2
<i>Fusarium</i> sp.	7	6	0	1	5	6	0	3	3

present in air water and soil and able to contaminate food if it is poorly processed (Żukiewicz-Sobczak *et al.*, 2015). *Aspergillus* species could be identified into *A. niger*, *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. ochraceus* and *A. terrus* with total percentages of 53 (37.9%), 25 (17.9%), 21 (15%), 5 (3.5%), 5 (3.5%) and 8 (5.7%), respectively (Table 3). The finding is in agreement with Darwish *et al.* (2016) who isolate *A. niger* and *A. flavus* from chicken meat samples retailed in Egypt and Zakki *et al.* (2017) who detected *A. niger*, *A. fumigates* and *A. flavus* in chicken meat collected from Pakistan.

3.3 Proteolytic and lipolytic activity of isolated mould

The ability of isolated mould to exhibits their proteases and lipases were examined, all *Aspergillus* species have the ability to produce proteases and lipases between high, moderate and weak degree except *A. parasiticus*. Furthermore, proteolytic and lipolytic activity was detected in all examined *Penicillium*, *Alternaria*, *Rhizopus*, *Mucor* species (Table 4). Certain mould strains of *Penicillium* and *Mucor* isolated from cold meats have ability to produce proteolytic and lipolytic activity against meat proteins and lipid both *in vitro* and in processed meat (Trigueros *et al.*, 1995; Toledo *et al.*, 1997). Additionally, the proteolytic and lipolytic activity previously detected in 52.70% and 94.59% of mould isolated from dry-cured ham (Alapont

et al., 2015); 82.4% and 88.9% of examined mould isolated from meat products have proteolytic and lipolytic activity (Habashy *et al.*, 2019). The fungi that produce proteolytic and lipolytic enzymes have two ways, the first good directions; play an important role in the appearance of favourable flavour to the consumer of some meat products as dry-cured ham and sausage (Flores *et al.*, 1997; Marušić *et al.* 2011). The worse direction, in frozen meat and chicken, leading to depletion in the nutritional quality of chicken products and changes in sensory characteristics (Wigmann *et al.*, 2015).

3.4 Aflatoxin residues in examined samples

Total aflatoxin residues estimated in the examined burger (9.21±1.12 µg/kg) and liver (8.79±14 µg/kg) was significantly higher (P<0.05) than other examined samples (Table 5). Parallel reports of aflatoxin occurrence in marketed poultry meat and giblets, 38% of broiler chicken meat samples were contaminated with aflatoxin the highest level was found in the liver 3.40±1.01 µg/kg in Pakistan (Iqbal *et al.*, 2014). In Mozambique, the level of contamination with aflatoxin B1 was 39% (0.57–3.80 µg/kg) for liver and 13.8% (0.68 - 2.12 µg/kg) for gizzard (Sineque *et al.*, 2017). Much lower aflatoxins contamination were reported in Egypt by Abd-Elghany and Sallam (2015) they detected 1.12±0.10 and 3.22±0.43 µg/kg for beef luncheon and

Table 5. Aflatoxin residues ($\mu\text{g}/\text{kg}$) of examined chicken meat, giblets and products in comparison with legal limits ($n = 20$).

	Breast	Thigh	Liver	Heart	Gizzard	Burger	Luncheon
Prevalence	4 (20%)	3 (15%)	11 (55%)	5 (25%)	4 (20%)	13 (65%)	7 (35%)
Minimum	0.12	0.16	2.13	0.13	0.14	2.84	1.16
Maximum	2.66	3.16	16.12	2.13	3.16	15.11	6.38
Mean \pm SD	1.49 \pm 0.53 ^b	1.89 \pm 0.89 ^b	8.79 \pm 14 ^a	1.64 \pm 0.38 ^b	2.3 \pm 0.72 ^b	9.21 \pm 1.12 ^a	4.12 \pm 0.68 ^{ab}
Within PL 20*	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)
Exceeded PL20*	0	0	0	0	0	0	0
Within PL4 [#]	20(100%)	20(100%)	11(55%)	20(100%)	20(100%)	9(45%)	16(80%)
Exceeded PL4 [#]	0	0	9(45%)	0	0	11(55%)	4(20%)

Values are presented as mean \pm SD. Values with different superscripts within each row are significantly different ($P < 0.05$).

*FDA (2000), [#]FAO (2004).

beef burger, respectively. While higher residue level (11.10 $\mu\text{g}/\text{kg}$) was detected in beef luncheon (Ismail and Zaky, 1999) and much higher residue (150 $\mu\text{g}/\text{kg}$) was obtained in kubeba (Aziz and Youssef, 1991) in Egypt. Conversely, much lower aflatoxins (0.05 $\mu\text{g}/\text{kg}$) were obtained in French delicatessen meats (Sirot *et al.*, 2013). The high level of aflatoxin residues in burger samples may be due to many sources of mould contamination, which could be introduced from the direct growth of moulds in the surface of meat products, or indirectly from non-meat additives that were previously contaminated with aflatoxin, such as spices and cereals, which are used in the formulation of chicken products as a main ingredient (European Commission, 2002). Moreover, higher aflatoxin levels in the liver are attributed to the role of the liver in detoxification of aflatoxin, to protect the body against their toxic effects (Fouad *et al.*, 2019).

Human all over the world may be exposed to potential health hazards due to consumption of mycotoxin, Maximum residue limit for aflatoxins in commodities have been assessed worldwide. The maximum threshold limit of 20 $\mu\text{g}/\text{kg}$ for aflatoxins in foods has been established (FDA, 2000), while a limit of 4 $\mu\text{g}/\text{kg}$ has been established for foods (FAO, 2004). On holding comparisons for aflatoxin with the established regulation of Food and Drug Administration (FDA, 2000) we found all examined samples (100%) within the maximum permissible limits (20 $\mu\text{g}/\text{kg}$). Meanwhile, comparison with a permissible limit of 4 $\mu\text{g}/\text{kg}$ set by the Food and Agriculture Organization (FAO, 2004) we found 9/20 (45%), 11/20 (55%) and 4/20 (20%) of liver, burger and luncheon, respectively (Table 5). It is, therefore, possible that chicken meat, giblets, and products tested may exist as a hazard to consumers and aflatoxin levels must be examined more broadly to protect human health.

4. Conclusion

Chicken meat cuts, giblets and products

contaminated with proteolytic and lipolytic mould which affect the quality in addition to the occurrence of aflatoxin residues that constitutes a public health hazard for consumer. So following the steps of the food safety management system can be a good solution to maintain the health and safety of the consumer.

Conflict of interest

The authors declare no conflict of interest.

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